

PB86-230844

Treatability of Water
Soluble Azo Dyes by the
Activated Sludge Process

(U.S.) Environmental Protection Agency
Cincinnati, OH

Aug 86



U.S. Department of Commerce
National Technical Information Service
NTIS

TREATABILITY OF WATER SOLUBLE AZO DYES
BY THE ACTIVATED SLUDGE PROCESS

by

Glenn M. Shaul*
Richard J. Lieberman**
Clyde R. Dempsey*
and
Kenneth A. Dostal*

*Industrial Wastes and Toxics Technology Division
Water Engineering Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

**Department of Civil & Environmental Engineering
University of Cincinnati
Cincinnati, Ohio 45221

WATER ENGINEERING RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OH 45268

TECHNICAL REPORT DATA		
(Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/D-86/167	2.	3. RECIPIENT'S ACCESSION NO. PB86 230844/AS
4. TITLE AND SUBTITLE Treatability of Water Soluble Azo Dyes by the Activated Sludge Process	5. REPORT DATE August 1986	
	6. PERFORMING ORGANIZATION CODE	
7. AUTHOR(S) G.M. Shaul, R.J. Lieberman, C.R. Dempsey and K.A. Dostal	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Water Engineering Research Laboratory U.S. EPA 26 W. St. Clair St. Cincinnati, OH 45268	10. PROGRAM ELEMENT NO.	
	11. CONTRACT/GRANT NO.	
12. SPONSORING AGENCY NAME AND ADDRESS Water Engineering Research Laboratory U.S. EPA 26 W. St. Clair St. Cincinnati, OH 45268	13. TYPE OF REPORT AND PERIOD COVERED Research 05/85 - 07/86	
	14. SPONSORING AGENCY CODE EPA/600/14	
15. SUPPLEMENTARY NOTES Project Officer: Glenn M. Shaul, WERL.		
16. ABSTRACT The U.S. Environmental Protection Agency's (EPA) Office of Toxic Substances (OTS) evaluates submissions to the Premanufacture Notification process under section 5 of the Toxic Substances Control Act. Azo dyes constitute a significant portion of these submissions. OTS is concerned because some of the dyes, dye precursors or their degradation products such as aromatic amines, which are also dye precursors, have been shown to be, or are suspected to be, carcinogenic. Therefore, EPA's Water Engineering Research Laboratory, Office of Research and Development has an ongoing study to determine the fate of specific water soluble azo dye compounds in the activated sludge process (ASP). The objective of this study is to dose pilot plant ASP systems with various water soluble azo dyes and monitor each dye compound through the system, analyzing both liquid and sludge samples. The fate of the parent dye compound is assessed via mass balance calculations. From these data, it is possible to determine if the compound was removed by adsorption, apparent biodegradation or not removed at all. This report presents results to date (7/86) from this study which is being conducted at EPA's Test and Evaluation Facility in Cincinnati, Ohio.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 22
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

TREATABILITY OF WATER SOLUBLE AZO DYES
BY THE ACTIVATED SLUDGE PROCESS

by

G.M. Shaul, R.J. Lieberman, C.R. Dempsey and K.A. Dostal

The U.S. Environmental Protection Agency's (EPA) Office of Toxic Substances (OTS) evaluates Premanufacture Notification (PMN) submissions under section 5 of the Toxic Substances Control Act (TSCA). Azo dyes constitute a significant portion of these submissions. Generally, azo dyes contain between one and three azo linkages (-N=N-), linking phenyl and naphthyl radicals that are usually substituted with some combination of functional groups including: amino (-NH₂); chloro (-Cl); hydroxyl (-OH); methyl (-CH₃); nitro (-NO₂) and sulfonic acid, sodium salt (-SO₃Na). OTS is concerned because some of the dyes, dye precursors and/or their degradation products such as aromatic amines, which are also dye precursors, have been shown to be, or are suspected to be, carcinogenic¹. Therefore, EPA's Water Engineering Research Laboratory, Office of Research and Development has an ongoing study to determine the fate of specific azo dye compounds in the activated sludge process (ASP).

This is being approached by dosing the feed to pilot ASP systems with various water soluble azo dyes and monitoring each dye compound through the system, analyzing both liquid and sludge samples. The fate of the parent dye compound is assessed via mass balance calculations. From these data, it is possible to determine if the compound is removed by adsorption, apparent biodegradation or not removed at all. This report presents results to date (7/86) from this study which is being conducted at EPA's Test and Evaluation Facility in Cincinnati, Ohio.

LITERATURE REVIEW

Limited work on aerobic biological treatment systems has been done regarding the fate of specific dye compounds in industrial or industrial/municipal wastewaters. Most of the research on aerobic systems has utilized synthetic wastewater which allows use of simplified analytical methodology but this does not represent the variability inherent in actual wastewaters. However, much of what has been done is still useful in predicting the fate of specific compounds in these treatment systems.

Straley² in his review of the literature stated that, "...dyes are generally recalcitrant to biodegradation since one of the important factors in selecting a dye is its resistance to oxidation." Although aerobic biodegradation may not be likely,

some researchers have shown azo dyes to be slowly biodegraded under aerobic conditions^{3,4,5}. However, the primary removal mechanism of dyes from wastewater treated by activated sludge seems to be adsorption onto biological sludge solids^{6,7}.

Tincher⁸ studied the removal of acid and disperse dyes (although not all dyes were of the azo type) in a synthetic industrial wastewater simulating a carpet dyeing process wastewater. Generally, he found wide fluctuations in removal of these dyes and speculated that when removal did occur the removal mechanism was probably adsorption onto sludge rather than biodegradation. He concluded that carbon adsorption in conjunction with biological treatment appeared to be a particularly effective treatment system for the removal of dyes from wastewaters.

Wuhrmann, et al.⁹ stated that permeation of dyes through the cell membrane is the primary rate limiting step in the microbial decolorization of azo dyes; therefore, sulfonic acid substitution (causing increased water solubility) seems to be an effective inhibitor of permeation.

EXPERIMENTAL PROGRAM

This paper describes work that used screened raw wastewater from the Greater Cincinnati Mill Creek Sewage Treatment Plant as the influent to three pilot-scale activated sludge biological treatment systems (two experimental and one control) operated in parallel. Each system consisted of a primary clarifier (33 liters), complete-mix aeration basin (200 liters) and a secondary clarifier (32 liters). The systems were designed to operate at variable flowrates with all flows controlled by variable speed peristaltic pumps. Adjustable weirs allow variation of retention times and overflow rates in both primary and secondary clarifiers. Wasting of primary sludge was performed manually while mixed liquor wasting from the aeration basin for control of the solids retention time (SRT) was performed automatically.

Each water soluble dye was dosed as commercial product to the two experimental systems operated in parallel at targeted active ingredient doses of 1 and 5 mg/L of influent flow (henceforth referred to as the low and high spike systems, respectively). Since it was desirable to spike the dyes into actual wastewaters, it was important that realistic dosages be selected. Discussions with OTS and representatives from a trade association, the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry resulted in selection of these dosages. The principal focus of this work is on the ASP and as such the primary sludge was not sampled. Table I presents a summary of the average operating conditions of the pilot plant systems.

TABLE I. SUMMARY OF OPERATING CONDITIONS

Parameter	Value
Influent Flow Rate, L/d	720
Primary Sludge Flow Rate, L/d	6
Primary Effluent Flow Rate, L/d	714
Mixed-Liquor Wastage Flow Rate, L/d	67
Secondary Effluent Flow Rate, L/d	647
Solids Retention Time, days	2.7
Hydraulic Retention Time, days	0.28
Dissolved Oxygen, mg/L	2.0-4.0
Target Influent Spike Dosages, mg/L	
Low	1
High	5
Influent pH, pH units	7.0-8.0
Aeration Basin Temperature °C	21-25

Before each intensive data collection phase, dye recovery studies were conducted using organic free water, influent wastewater and mixed liquor. These studies were run in duplicate and each recovery study was repeated at least once in order to ensure that the compound could be extracted from these samples. Purified dye standards were analytically prepared from the commercial dye product by repeated recrystallization.

The primary effluent (PE) and activated sludge effluent (ASE) samples were prepared as follows:

- Step 1. One liter samples were filtered through 934AH.
- Step 2. Filtrate was passed through a XAD-2 column and then soaked for 16 hours in ammonia-acetonitrile solution.
- Step 3. Resin and filter paper were Soxhlet extracted with ammonia-acetonitrile for 16 hours.
- Step 4. Extract was concentrated and brought up to 50ml volume with methanol/dimethylformamide.

The mixed liquor (ML) samples were prepared as follows:

- Step 1. One liter sample was filtered through 0.45 micron pore size filter. The filtrate (SOL) was then prepared similar to the PE and ASE samples (Steps 2, 3 and 4) except the filter paper was not used in the extraction.
- Step 2. The suspended residue (RES) and the filter paper were soaked for 16 hours in ammonia-acetonitrile solution then Soxhlet extracted with ammonia-acetonitrile for 16 hours.

Step 3. Each extract was concentrated and brought up to 50 ml volume with methanol/dimethylformamide.

All extracted samples were analyzed by high performance liquid chromatography (HPLC) with an ultraviolet-visible detector.

The targeted range of recovery from all samples was 80 to 120 percent. If recovery analyses were within this range, then it was assumed that little, if any, chemical transformation was occurring. Acceptable recoveries were achieved for all samples (liquids and sludges), and as such, all data reported herein are as measured and have not been corrected for recoveries. All samples were analyzed by the University of Cincinnati's Kettering Laboratory of the College of Medicine.

All systems were operated for at least three times the solids retention time (SRT) to ensure acclimation prior to initiation of data collection. During the intensive data collection period the following analytical tests were performed:

- o Total suspended solids (SS) analyses on the influent (INF), PE, ML and ASE samples. All samples were analyzed using EPA approved method 160.2-1¹⁰.
- o Specific dye analyses on the PE, ML and ASE samples. On occasion, the INF samples were analyzed to ensure that targeted dosages were maintained. No standard method exists for these analyses, however a method developed by Tincher⁸ and Dickson¹¹, and described above, was used.

All samples were 24-hour composites made up of six grab samples collected every four hours and stored at 4°C. Each test run was sufficiently long to allow at least six sets of samples collected over three to five weeks (after acclimation) for the specific dye analyses. If acclimation appeared to be continuing during the data collection phase, the intensive data collection period was extended accordingly.

In addition to the pilot plant studies, adsorption isotherms were developed using activated sludge solids as the adsorbent. Purified dyes prepared from the commercial product were used in these studies in order to minimize interferences from the impurities of the commercial formulations. These studies were conducted with sludge solids obtained from the control pilot plant system which did not receive a dye spike. In order to prevent potential biodegradation from influencing the adsorption measurements, the biological solids were inactivated by freeze-drying of the solids with subsequent desiccation of the dry biomass in an oven for a minimum of three hours at 105°C. This

procedure was reported to be the simplest method for inactivation of the biomass while generally retaining the structural integrity of the biomass¹². Flocculation and settling properties of this freeze-dried, desiccated biomass upon rehydration resembled viable activated sludge solids. In addition, photomicrographs of this material revealed the presence of organisms with apparent external structures intact.

Freeze-dried, desiccated biomass was utilized in kinetic studies to determine the contact time required for the test dyes and sludge solids to reach equilibrium. Previous studies have reported contact times of 40 to 120 minutes⁶, 30 minutes⁷ and 20 minutes¹³ for some azo dyes. However, 24 hours was chosen as the standard contact time for all dyes investigated in this study since the kinetic experiments revealed that although for some dyes equilibrium was reached within one or two hours, other dyes required six or more hours of contact between the test dye and biomass for adsorption equilibrium to be attained.

Adsorption isotherm experiments were performed once the equilibrium contact time was established. A typical experimental set-up consisted of a series of stoppered glass vessels which contained increasingly greater concentrations (typical range was 0.5 to 8.0 g/L solids concentrations) of the inactivated sludge solids which were rehydrated in buffered distilled water. A known concentration of dye was spiked into each vessel and the contents of each were well mixed for 24 hours using a magnetic stirring apparatus. A quality control sample included in this set-up consisted of a dye blank (no sludge solids) to assess potential changes in soluble dye concentration due to dye molecules adhering to the inside walls of the container or photodegradation. Another quality control sample was obtained from a vessel containing sludge solids in buffered water which did not receive the dye spike. This was then used to assess potential analytical interferences originating from the biomass. The contents of all vessels were sampled at the end of the test. These samples were centrifuged at 10,000 revolutions per minute for ten minutes and the centrate was subsequently filtered through a 0.45 micron pore size filter.

High performance liquid chromatography (HPLC) was used to analyze for the residual dye in the sample filtrates. The amount of residual dye was then utilized with the amount of biomass in each vessel to calculate an adsorption isotherm according to the Freundlich relationship. This is an empirically derived¹⁴ relationship with the general form:

$$X/M = K C_e^{1/n} \quad (1)$$

where:

X = amount of solute (dye) adsorbed, mg/L

M = concentration of adsorbent (sludge solids), g/L

X/M = amount of dye adsorbed per unit mass of sludge solids,
mg/g

C_e = equilibrium concentration of dye in solution, mg/L

K, n = empirical constants

The linear form of the Freundlich model may be illustrated graphically with a plot of X/M versus C_e on log-log paper. A line of best fit can be calculated using least squares regression analysis. The slope of this line ($1/n$) indicates adsorption intensity while the constant K indicates adsorption capacity in terms of the amount of test compound adsorbed per unit mass of adsorbent at a residual (soluble) concentration of test compound equal to 1 mg/L.

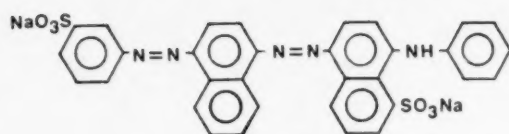
Other adsorption models could have been evaluated. For example, Langmuir's equation and the Brunauer-Emmett-Teller (BET) equation are commonly utilized in adsorption studies¹⁵. However, Dohanyos, et al⁶ applied all three models to data for 22 textile dyes and found the Freundlich relationship to be the most suitable for 18 of these dyes with activated sludge solids as the adsorbent.

RESULTS AND DISCUSSION

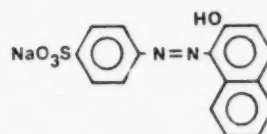
Nine water soluble, acid and direct azo dyes have been studied in both the pilot-scale ASP systems and the adsorption isotherm determinations and are listed below by Colour Index¹⁶ name and number with Figure 1 presenting the chemical structure for each.

Name	Number	Name	Number
C.I. Acid Blue 113	26360	C.I. Acid Red 151	26900
C.I. Acid Orange 7	15510	C.I. Acid Red 337	-----
C.I. Acid Orange 10	16230	C.I. Acid Yellow 151	13906
C.I. Acid Red 1	18050	C.I. Direct Violet 9	27885
C.I. Acid Red 88	15620		

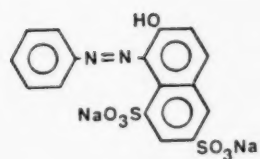
The possible removal mechanisms for a dye compound in the ASP system include adsorption, biodegradation, chemical transformation, photodegradation, and air stripping. During the spike recovery studies for each dye, several wastewater and sludge



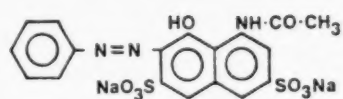
C.I. ACID BLUE 113



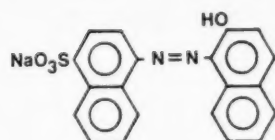
C.I. ACID ORANGE 7



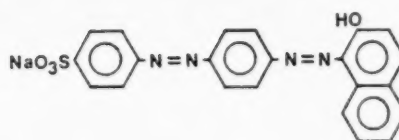
C.I. ACID ORANGE 10



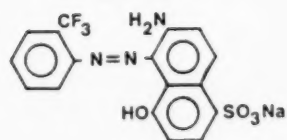
C.I. ACID RED 1



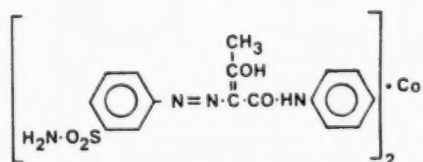
C.I. ACID RED 88



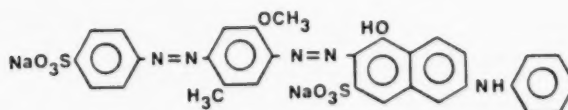
C.I. ACID RED 151



C.I. ACID RED 337



C.I. ACID YELLOW 151



C.I. DIRECT VIOLET 9

samples from the control ASP were spiked and recovery was assessed. Recovery for all dyes was within the targeted range of 80 to 120 percent; thus, it appeared that little or no chemical transformation occurred for any dye due to contact with the variable wastewater and/or sludge matrix. In addition, photodegradation potential was investigated with each dye added to distilled/deionized water and no measurable decrease in concentration was observed for samples held in light for at least two weeks. Moreover, the estimated Henry's law constant for each dye tested is less than 10^{-15} atm-m³/mol and as such, air stripping is very unlikely¹⁷. Therefore, adsorption and/or biodegradation appeared to be the only possible removal mechanisms.

In order to determine the fate of the parent dye compound in the ASP systems and to allow a comparison between the adsorption isotherm data and the ASP data, it was initially assumed that no biodegradation occurred and mass balance calculations were made. The mass balance calculations are presented below:

$$\text{DYE IN} = (\text{PE FLOW RATE, L/d}) (\text{PE DYE CONC., mg/L}) = \text{mg/d}$$

$$\text{DYE OUT 1} = (\text{DYE OUT VIA SECONDARY EFFLUENT})$$

$$\text{DYE OUT 1} = (\text{ASE FLOW RATE, L/d}) (\text{ASE DYE CONC., mg/L}) = \text{mg/d}$$

$$\text{DYE OUT 2} = (\text{DYE OUT VIA WASTING MIXED LIQUOR, WAS})$$

$$\begin{aligned} \text{DYE OUT 2} &= (\text{WAS FLOW RATE, L/d}) (\text{WAS SOL DYE CONC., mg/L}) \\ &+ (\text{WAS FLOW RATE, L/d}) (\text{WAS ADSORBED DYE CONC., mg/L}) = \text{mg/d} \end{aligned}$$

with

$$(\text{WAS ADSORBED DYE CONC.}) = (\text{WAS SS CONC., g/L}) (\text{RES DYE CONC., mg/g})$$

$$\text{DYE OUT} = (\text{DYE OUT 1}) + (\text{DYE OUT 2})$$

$$\text{DYE Recovered, \%} = (\text{DYE OUT/DYE IN}) \times 100$$

Table II presents the mean SS and dye concentrations calculated from the ASP raw data, while Table III presents the mass balance closure data calculated from the values in Table II.

Upon inspection of the data from Table II, it can be seen that wide fluctuations in dye removal (PE versus ASE) were measured. (Note that two runs were conducted for C.I. Acid Orange 7.)

From Table III, it can be seen that five of the nine dyes studied in the ASP systems had total mass balance closures within the 80 to 120 percent range. This indicates that there was little or no biodegradation of these dyes. The dyes were C.I. Acid Orange 10, C.I. Acid Red 1, C.I. Acid Red 337, C.I. Acid Yellow 151, and C.I. Direct Violet 9. C.I. Acid Orange 10 and C.I. Acid Red 1 had very little adsorption onto the sludge with the majority of each

TABLE II. Data Summary

	Primary Effluent (PE)			Secondary Effluent (ASE)			Waste Activated Sludge (WAS)			
	SS (mg/L)	Total Dye Conc. (mg/L)	Mass of Total Dye Per Day ¹ (mg/d)	SS (mg/L)	Total Dye Conc. (mg/L)	Mass of Total Dye Per Day ² (mg/d)	SS (mg/L)	SOL Dye Conc. ³ (mg/L)	Mass of SOL Dye per Day ⁴ (mg/d)	Mass of RES Dye per Day ⁵ (mg/d)
<u>C. I. Acid Blue 113 (n=5)*</u>										
Low Spike	190	0.84	600	7	0.07	45	1490	0.04	3	409
High Spike	220	4.55	3250	15	0.84	543	1150	0.44	29	1590
<u>C. I. Acid Orange 7</u>										
<u>Run 1 (n=6)</u>										
Low Spike	140	0.95	678	17	0.18	116	950	0.15	10	<1
High Spike	90	5.34	3810	21	0.24	155	910	0.14	9	<1
<u>Run 2 (n=10)</u>										
Low Spike	160	1.07	764	21	0.29	188	1010	0.22	15	<2
High Spike	220	5.57	3980	41	0.73	472	970	0.53	36	>1
<u>C.I. Acid Orange 10 (n=6)</u>										
Low Spike	180	0.96	685	15	1.01	653	1070	0.88	59	<1
High Spike	180	5.55	3960	8	5.49	3550	1140	4.94	331	>3
<u>C.I. Acid Red 1 (n=8)</u>										
Low Spike	190	0.90	643	25	0.88	569	1860	0.83	56	<1
High Spike	260	4.43	3160	12	4.48	2900	1690	4.42	296	>6

TABLE II. Data Summary (Cont.)

	Primary Effluent (PE)			Secondary Effluent (ASE)			Waste Activated Sludge (WAS)		
	SS (mg/L)	Total Dye Conc. (mg/L)	Mass of Total Dye Per Day ¹ (mg/d)	SS (mg/L)	Total Dye Conc. (mg/L)	Mass of Total Dye Per Day ² (mg/d)	SS (mg/L)	SOL Dye Conc. ³ (mg/L)	Mass of RES Dye per Day ⁴ (mg/d)
<u>C.I. Acid Red 88 (n=5)</u>									
Low Spike	140	0.68	486	14	0.02	13	910	0.01	4
High Spike	150	3.96	2830	22	0.01	<6	1300	0.01	7
<u>C.I. Acid Red 151 (n=6)</u>									
Low Spike	140	0.56	400	14	0.17	110	910	0.08	176
High Spike	150	3.64	2600	22	0.44	285	1300	0.17	1720
<u>C.I. Acid Red 337 (n=5)</u>									
Low Spike	190	1.13	807	7	0.93	602	1490	0.68	115
High Spike	220	5.06	3610	13	4.40	2850	1150	3.61	326
<u>C.I. Acid Yellow 151 (n=8)</u>									
Low Spike	190	0.67	478	25	0.48	311	1860	0.26	57
High Spike	260	4.05	2890	12	4.08	2640	1690	2.66	488
<u>C.I. Direct Violet 9 (n=6)</u>									
Low Spike	220	0.78	557	16	0.49	317	1230	0.21	241
High Spike	260	4.71	3360	11	0.99	641	1480	0.38	2320

1 PE flow rate = 714 L/d

2 ASE flow rate = 647 L/d

3 SOL DYE = Concentration of soluble dye in filtrate

4 WAS flow rate = 67 L/d

5 RES Dye = Mass of dye adsorbed per mass (dry weight) of SS in the WAS

* n = number of samples

TABLE III. Mass Balance Summary

		Percent of Primary Effluent Dye Feed in:				Apparent Biodegradation*
		Activated Sludge	Waste Activated		ASE + WAS	
		Effluent	Sludge (WAS)			
		ASE	SOL	RES	TOTAL	
		%	%	%	%	%
<u>C.I. Acid Blue 113</u>						
	Low Spike	8	<1	68	76	24
	High Spike	17	1	49	67	33
<u>C.I. Acid Orange 7</u>						
Run 1	Low Spike	17	1	<1	19	81
	High Spike	4	<1	<1	4	96
Run 2	Low Spike	25	2	<1	27	73
	High Spike	12	1	<1	13	87
<u>C.I. Acid Orange 10</u>						
	Low Spike	95	9	<1	104	0
	High Spike	90	8	<1	98	0
<u>C.I. Acid Red 1</u>						
	Low Spike	88	9	<1	97	0
	High Spike	92	9	<1	101	0
<u>C.I. Acid Red 88</u>						
	Low Spike	3	<1	1	4	96
	High Spike	<1	<1	<1	<1	>99
<u>C.I. Acid Red 151</u>						
	Low Spike	28	1	44	73	27
	High Spike	11	<1	66	78	22
<u>C.I. Acid Red 337</u>						
	Low Spike	75	6	14	95	0
	High Spike	79	7	9	95	0
<u>C.I. Acid Yellow 151</u>						
	Low Spike	65	4	12	81	0
	High Spike	91	6	17	114	0
<u>C.I. Direct Violet 9</u>						
	Low Spike	57	3	43	103	0
	High Spike	19		69	89	0

*Calculated as $100 - (\text{ASE} + \text{WAS})$. When ASE + WAS was within 80-120% range, it was assumed that very nominal, if any, biodegradation occurred.

compound exiting the ASP in the secondary effluent stream. Both C.I. Acid Red 337 and C.I. Acid Yellow 151 were found to be partially adsorbed (9 to 17 percent) onto the sludge; however, most of these dyes was found in the secondary effluent.

The data in Table III show that only 4 to 27 percent and 1 to 4 percent of the influent dye could be accounted for in the ASE + WAS for C.I. Acid Orange 7 and C.I. Acid Red 88, respectively. Thus, it appears that both dyes underwent significant biological degradation.

C.I. Acid Blue 113, C.I. Acid Red 151 and C.I. Direct Violet 9 were strongly adsorbed (see WAS RES in Table II). From Table III, it can be seen that C.I. Direct Violet 9 had a mass balance closure within the 80 to 120 percent range. However, between 22 and 33 percent of the amount of C.I. Acid Blue 113 and C.I. Acid Red 151 entering the aeration basin could not be accounted for in the ASE + WAS. Thus, there may have been some limited degradation of these two dyes, since the preliminary recovery studies showed that these dyes could be recovered within the 80 to 120 percent range.

Based upon the data collected to date, it is evident that wide fluctuations in dye compound behavior in the activated sludge process does occur. Figure 2 presents a graphic representation of this range of behavior. Broad ranges, based upon ASP data collected to date, which may change with additional ASP data for other dyes, have been selected to depict low, moderate and high levels of adsorption and apparent biodegradation.

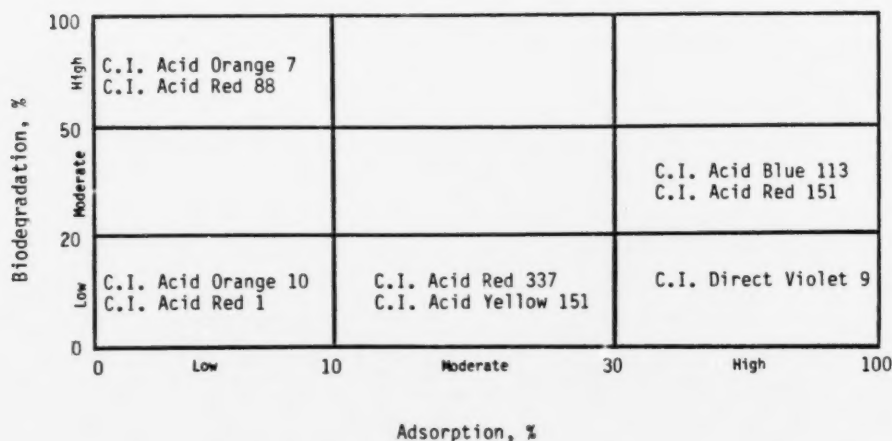


Figure 2. Summary of dye removal mechanisms

Table IV presents a summary of the adsorption isotherm results showing the adsorption constants (slope, $1/n$ and intercept, K) and the linear regression coefficient (r). These data can be presented graphically and Figure 3 presents the isotherm graph for C. I. Acid Yellow 151 as an example.

TABLE IV. Adsorption Isotherm Results

	Slope $1/n$	Intercept* K	Correlation Coefficient r
C. I. Acid Blue 113	1.2	6.7	0.952
C. I. Acid Orange 7	1.1	0.093	0.935
C. I. Acid Orange 10	**	**	**
C. I. Acid Red 1	**	**	**
C. I. Acid Red 88	0.77	4.1	0.973
C. I. Acid Red 151	1.3	7.6	0.824
C. I. Acid Red 337	1.3	0.67	0.952
C. I. Acid Yellow 151	1.5	1.3	0.956
C. I. Direct Violet 9	0.99	1.6	0.976

* When $C_e = 1$ mg/L

** Compound was found to be essentially non-adsorbable; constants could not be calculated.

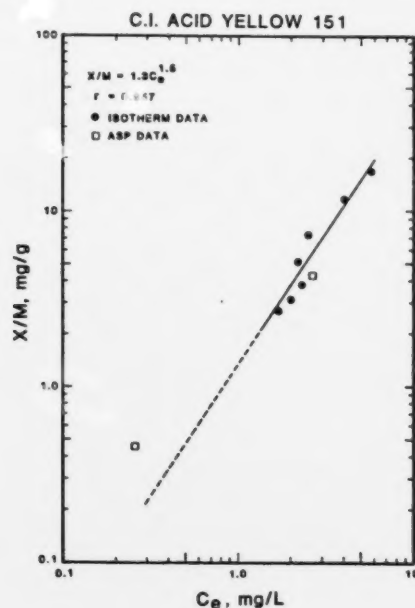


Figure 3. Adsorption Isotherm for C.I. Acid Yellow 151

The ASP data can be presented on an isotherm plot as well. The value for C_e is directly related to the SOL values from the ASP data, each representing the equilibrium concentration of dye in solution (mg/L). Similarly, the value of X/M is directly related to the RES values from the ASP data, each representing the amount of dye adsorbed per unit weight of sludge solids (mg/g).

If the ASP data fall on or near the calculated line of best fit from the isotherm, then one could conclude that the isotherm provided a good estimate of the dye removal due to adsorption in the ASP studies. In the case of C. I. Acid Yellow 151, it can be seen that a reasonably good relationship exists between the ASP data and the isotherm data.

Rather than showing several plots, a summary table can be developed to examine the relationship between the ASP data and the isotherm data. In order to do this, Equation 1 is used with data from Tables II and IV substituted into it. For example, for C. I. Acid Yellow 151, the isotherm constants are $1/n = 1.5$ and $K = 1.3$ resulting in the following:

$$X/M = (1.3)C_e^{1.5} \quad (2)$$

Thus, substituting the values for "SOL Dye Concentrations" in the WAS, which relates to C_e , for C. I. Acid Yellow 151 from Table II, one can calculate the value of X/M predicted by the isotherm test. For the high spike system it follows that:

$$X/M = 1.3 (2.66)^{1.5} \quad (3)$$

$$X/M = 5.64 \text{ mg/g}$$

A comparison of the predicted value to the measured value for "RES Dye Mass per SS Mass" from Table II shows 5.64 mg/g predicted versus 4.31 mg/g measured. Allowing that these tests are not directly equal since the test conditions and sludges are different, this comparison is still good, showing the isotherm test to be a good predictor for this compound. Table V summarizes these calculations for all compounds.

As can be seen from Table V, the predicted values for C. I. Acid Orange 10 and C. I. Acid Red 1 could not be calculated because the adsorption coefficients could not be calculated. It is clear, however, that the results from the isotherm were consistent with the ASP data; i.e., showing little or no adsorption onto the WAS for each compound. On the whole, the predicted versus measured values matched well for C. I. Acid Orange 7, C. I. Acid Red 88, C. I. Acid Red 337, and C. I. Acid Yellow 151. However, the predicted values were an order of magnitude off the measured values for C. I. Acid Blue 113, C. I. Acid Red 151, and C. I. Direct Violet 9 with the isotherm predicting much less adsorption than was measured. These differences cannot be explained at this time.

Table V. Comparison of ASP Data and Isotherm Predictions*

		C _e Measured (mg/l)	X/M Measured (mg/g)	X/M Calculated ⁺ (mg/g)
<u>C. I. Acid Blue 113</u>				
	Low Spike	0.04	4.10	0.14
	High Spike	0.44	20.54	2.50
<u>C. I. Acid Orange 7</u>				
Run 1	Low Spike	0.15	<0.02	0.01
	High Spike	0.14	<0.02	0.01
Run 2	Low Spike	0.22	<0.02	0.01
	High Spike	0.53	<0.02	0.05
<u>C. I. Acid Orange 10</u>				
	Low Spike	0.88	<0.02	**
	High Spike	4.94	<0.04	**
<u>C. I. Acid Red 1</u>				
	Low Spike	0.83	<0.01	**
	High Spike	4.42	0.05	**
<u>C. I. Acid Red 88</u>				
	Low Spike	0.02	0.10	0.21
	High Spike	0.01	0.08	0.12
<u>C. I. Acid Red 151</u>				
	Low Spike	0.08	2.89	0.28
	High Spike	0.17	19.80	0.76
<u>C. I. Acid Red 337</u>				
	Low Spike	0.68	1.15	0.41
	High Spike	3.61	4.08	3.55
<u>C. I. Acid Yellow 151</u>				
	Low Spike	0.26	0.46	0.17
	High Spike	2.66	4.31	5.64
<u>C. I. Direct Violet 9</u>				
	Low Spike	0.21	2.93	0.34
	High Spike	0.38	23.36	0.61

* Values reported as "less than" were used as the discrete number.

** Adsorption isotherm constants could not be generated for these dyes because they did not exhibit any measurable adsorption in the isotherm experiments. Therefore, the isotherm experiments for these dyes would predict X/M to be virtually zero with nearly all of the dye mass in the soluble phase.

+ Calculated using isotherm equation with C_e = WAS SOL DYE concentration from Table II.

CONCLUSIONS

Based upon the data presented, the following conclusions can be made:

- * C. I. Acid Orange 10 and C. I. Acid Red 1 appeared to be very nominally, if at all, adsorbed and not biodegraded.
- * C. I. Acid Red 337 and C. I. Acid Yellow 151 appeared to be moderately adsorbed but not biodegraded.
- * C. I. Direct Violet 9 appeared to be strongly adsorbed but not biodegraded.
- * C. I. Acid Blue 113 and C. I. Acid Red 151 appeared to be strongly adsorbed and possibly partially biodegraded.
- * C. I. Acid Orange 7 and C. I. Acid Red 88 appeared to be very nominally, if at all, adsorbed and significantly biodegraded.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Kevin J. Mulligan, University of Cincinnati, for his and his staff's work in the analyses of all samples. Glenn M. Shaul, Clyde R. Dempsey, and Kenneth A. Dostal are all Environmental Engineers with U. S. E.P.A.'s Office of Research and Development, Water Engineering Research Laboratory, Cincinnati, Ohio 45268. Richard J. Lieberman is a graduate student in the University of Cincinnati's Civil and Environmental Engineering Department.

NOTICE

This document has been reviewed in accordance with U. S. Environmental Protection Agency policy and approved for public distribution and/or publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Helemes, C. T., et al, "A Study of Azo and Nitro Dyes for the Selection of Candidates for Carcinogen Bioassay", Journal of Environmental Science and Health, Volume A19, No. 2, pp. 97-231 (1984).
2. Straley, J. P., "Selected Bacterial Cultures for Textile Wastewaters" in American Dyestuff Reporter, Sept. and Oct., 1984.
3. Kulla, H. G., "Aerobic Bacterial Degradation of Azo Dyes", in Microbial Degradation of Xenobiotics and Recalcitrant Compounds, Academic Press, Inc., London (1981).
4. Pratt, H. D., "A Study of the Degradation of Some Azo Disperse Dyes in Waste Disposal Systems", M. S. Thesis, Georgia Institute of Technology, (1968).
5. Anliker, R. Clarke, E. A. and Moser, P., "Use of the Partition Coefficient as an Indicator of Bioaccumulation Tendency of Dyestuffs in Fish", Chemosphere, Vol. 10, No. 3, pp. 263-274 (1981).
6. Dohanyos, M., Madera, V., and Sedlacek, M., "Removal of Organic Dyes by Activated Sludge", Progress in Water Technology, Vol. 10, Nos. 5/6, pp. 559-575, Pergamon Press (1978).
7. Hitz, H. R., et al., "The Adsorption of Dyes on Activated Sludge", Journal of the Society of Dyers and Colorists, 94, pp. 71-76 (Feb. 1978).
8. Tincher, W. C., "Survey of the Coosa Basin for Organic Contaminants from Carpet Processing", Contract No. 3-27-630 for the Environmental Protection Division, Department of Natural Resources, State of Georgia (1978).
9. Wuhrmann, K., Mechsner, K., and Kappeler, T., "Investigation on Rate-Determining Factors in the Microbial Reduction of Azo Dyes", European Journal Applied Microbiology and Biotechnology, 9, pp. 325-338 (1980).
10. "Methods for Chemical Analysis of Water and Wastes", EPA-600/4-79-020, Environmental Monitoring and Support Laboratory, U.S. EPA (1979).
11. Dickson, D. N., "The Effectiveness of Biodegradation in the Removal of Acid Dyes and Toxicity from Carpet Dyeing Wastewater", M. S. Thesis, Georgia Institute of Technology (1981).

12. Blackburn, J. W., et al, "Prediction of the Fates of Organic Chemicals in Activated Sludge Wastewater Treatment Processes", EPA 600/2-85-102, U.S. EPA (1985).
13. Nakaoka, M., Tamura, S., Maeda, Y., and Azumi, T., "Adsorption of Synthetic Dyes on Activated Sludge", Sen i Gakkaishi, 30(2), pp. 64-74 (1983).
14. Freundlich, H., Kapillarchemie, Akademische Verlagsgesellschaft M.B.H., Leipzig, East Germany (1922).
15. Morris, J. C. and Weber, W. J., Jr., "Adsorption of Biochemically Resistant Materials from Solution, No. 1, "Report No. 999-WP-11, U.S. Department of Health, Education and Welfare, Public Health Service, Division of Water Supply and Pollution Control, (1964).
16. Colour Index, Revised 3rd Edition, Seven Volumes, published by The Society of Dyers and Colourists (1982).
17. Lyman, W., Reehl, W., and Rosenblatt, D., Handbook of Chemical Property Estimation Methods, McGraw Hill Book Company, New York (1982).